

~~#5~~

Sorting by pools

Master

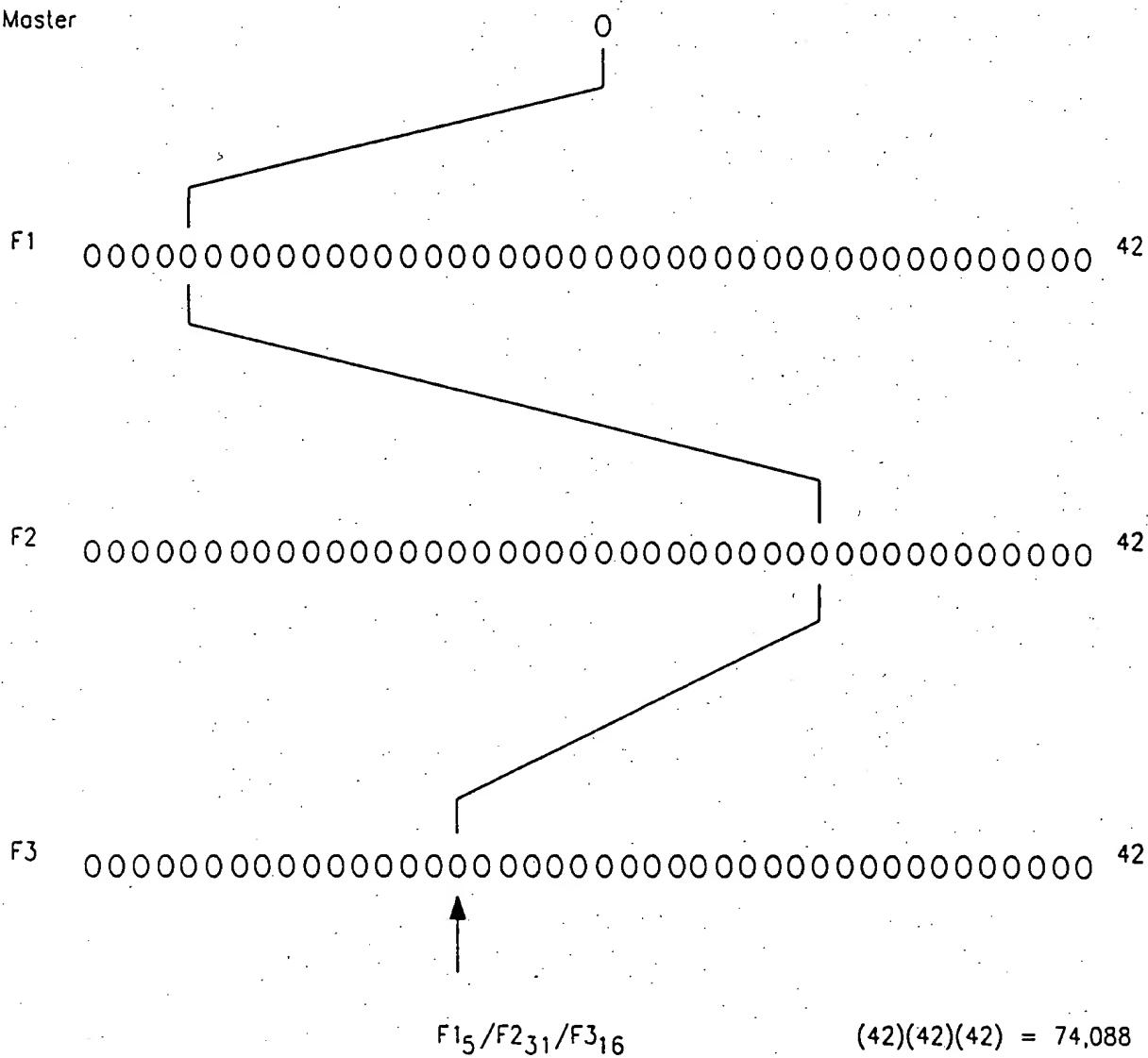


FIG. I

Sorting by pools: Decreasing pool diversities

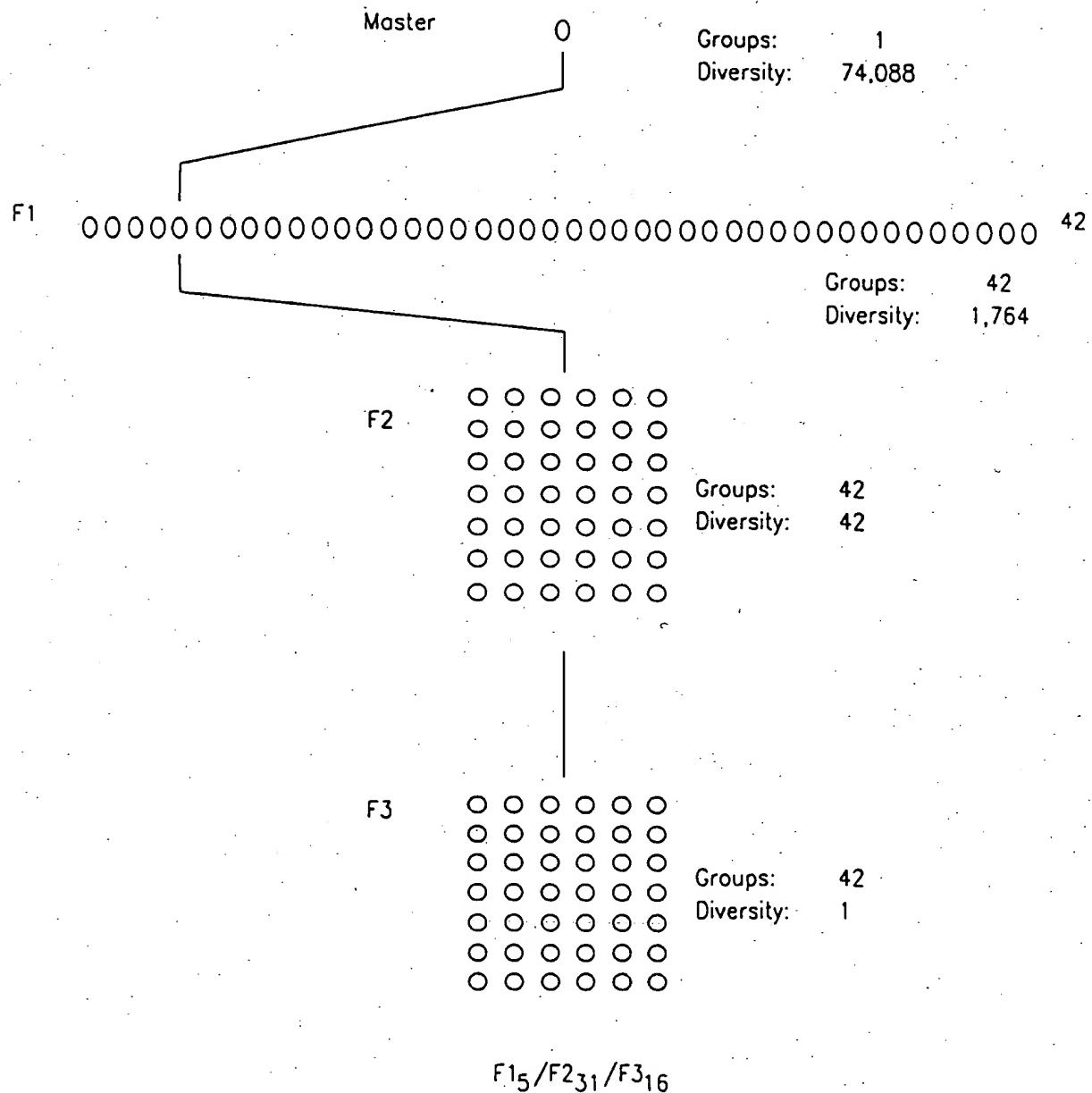


FIG. 2

Title: **COLLECTIONS OF BINDING PROTEINS AND TAGS AND USES THEREOF FOR NESTED SORTING AND HIGH THROUGHPUT SCREENING.**
 Applicant: Ault-Richter *et al.*
 Serial No. 09/910,120 Filed: July 18, 2001
 Our Docket No.: 25885-1751

Sorting by pools: Screening large diversity libraries

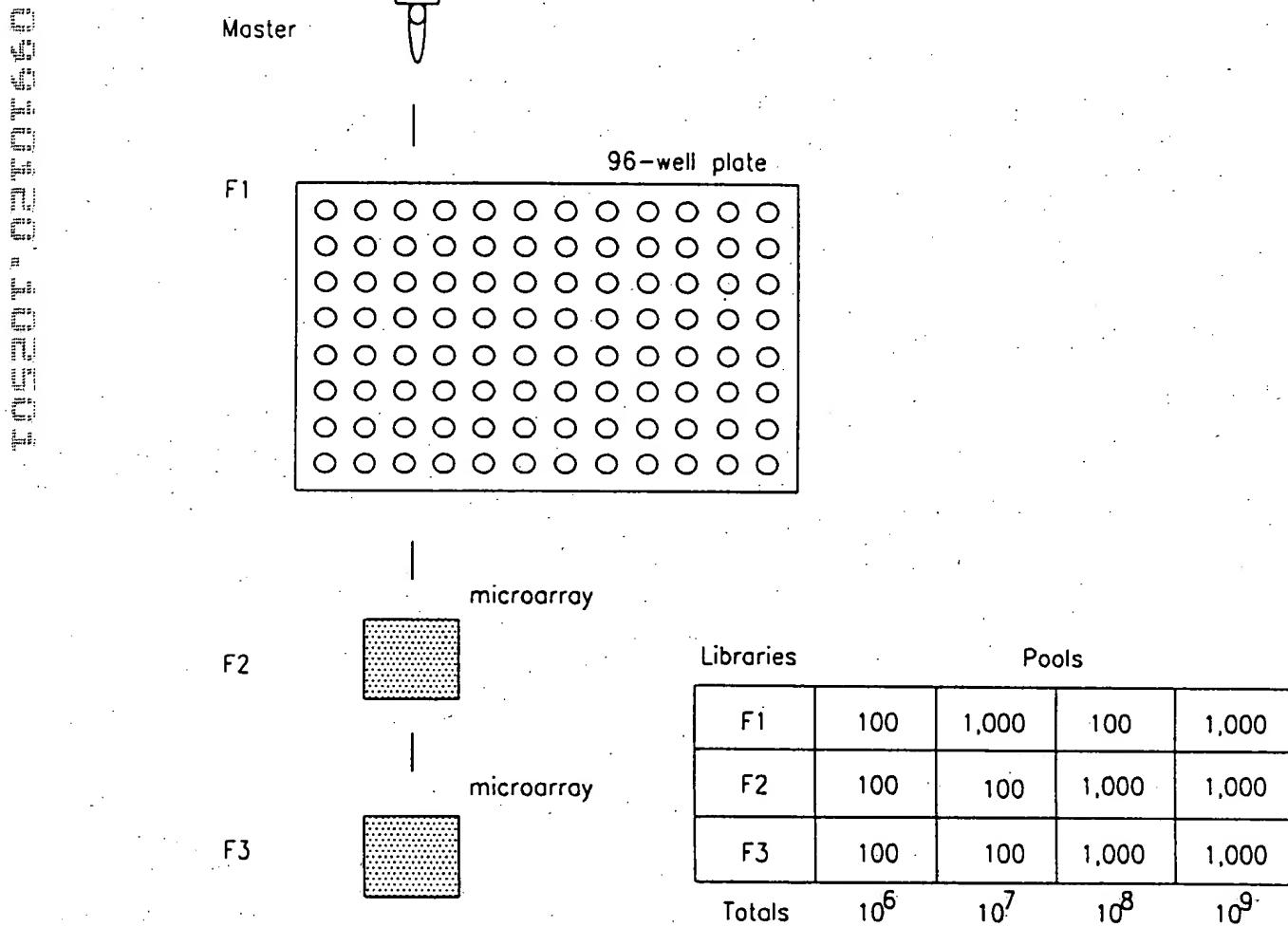


FIG. 3

Searching a mutation library

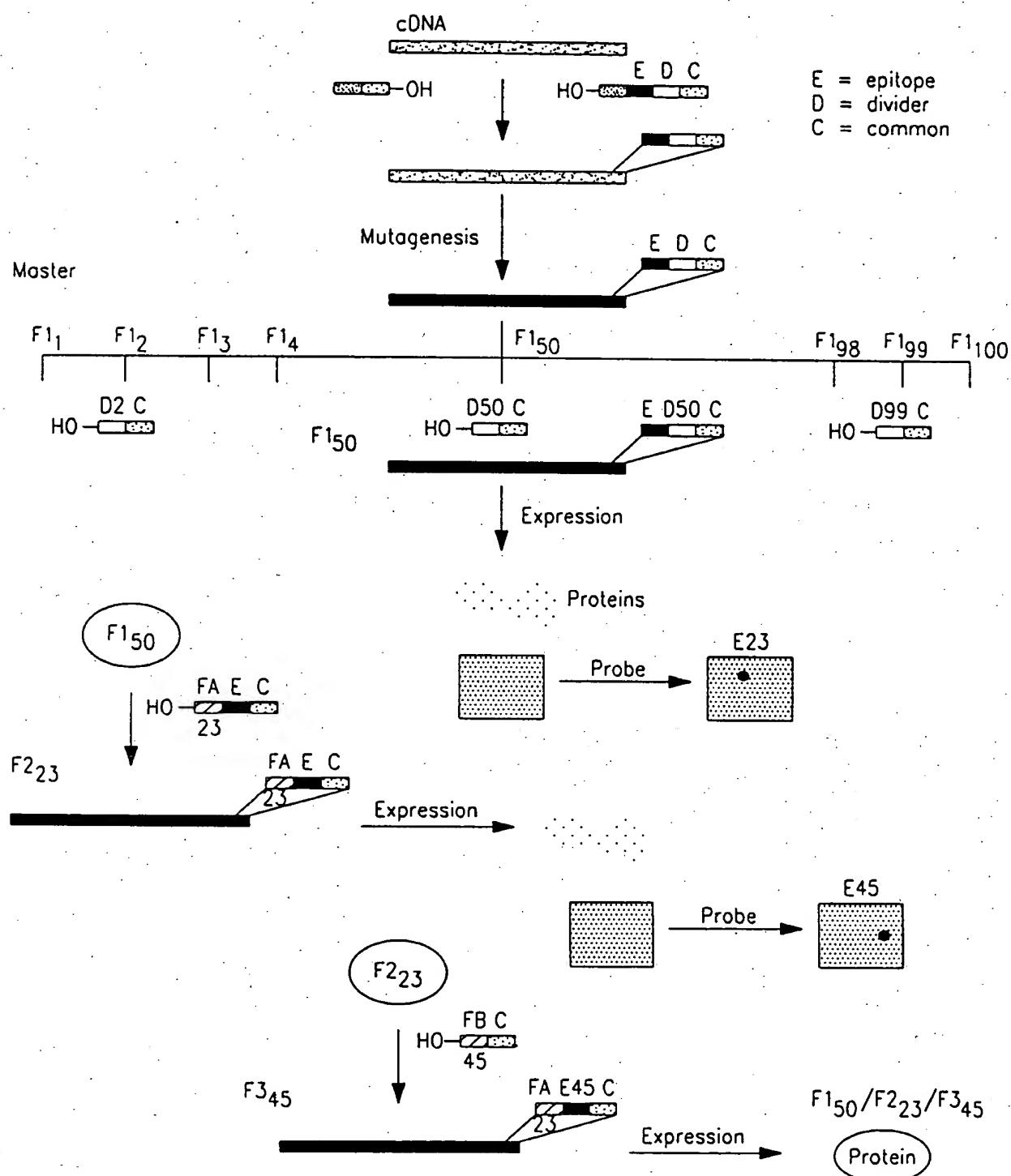
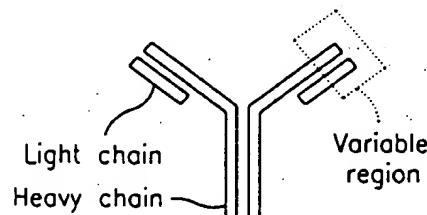


FIG. 4

Making a recombinant antibody library



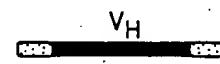
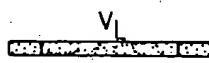
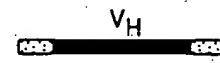
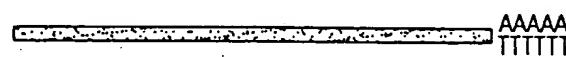
Spleen cells or PBLs



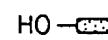
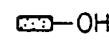
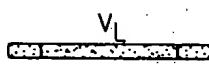
mRNA



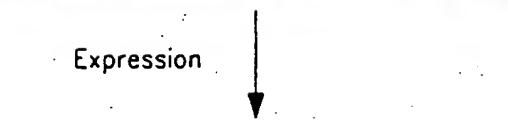
cDNA



Linker



Expression

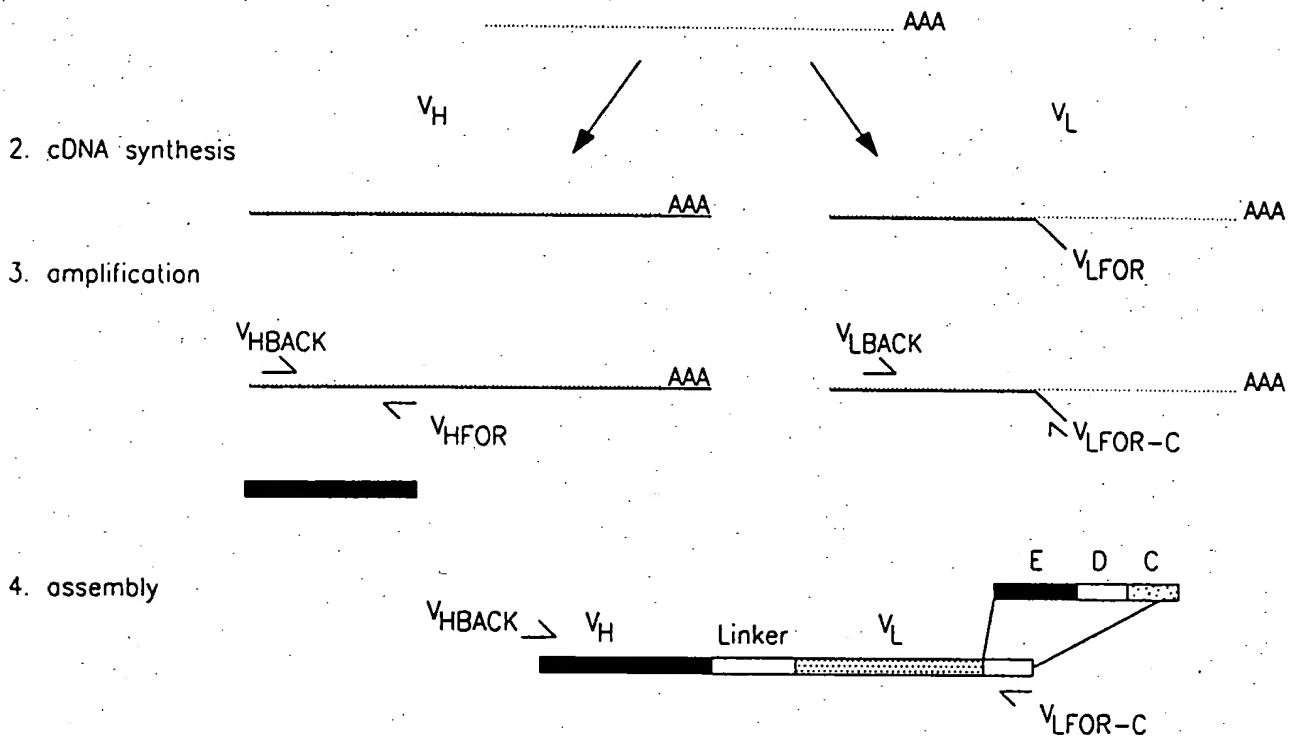


Antibodies

FIG. 5

Creating the master antibody library: Primer incorporation

1. mRNA purification from spleen or PBLs



V_H Primers

Oligo dT

HO-TTTTTTTT(T)_n
 3' 5'

V_HBACK

V_H back
 OH
 5' 3'

V_HFOR

J_H for
 OH
 3' 5'

V_L Primers

V_LFOR

J_{kappa} for E D C
 OH
 3' 5'

V_LBACK

V_{kappa} back
 OH
 5' 3'

V_LFOR-C

C
 OH
 3' 5'

FIG. 6

Creating the master antibody library: Linker addition

1. mRNA purification from spleen or PBLS

..... AAA

V_H

AAA

V_L

AAA

2. cDNA synthesis

..... AAA

V_LFOR

3. amplification

V_HBACK

AAA

V_LBACK

AAA

V_HFOR

Digest end and mix with V_LLinkers

V_LLinkers

Ligate and amplify

V_LBACK

V_LFOR-C

4. assembly

V_HBACK

V_H

Linker

V_L

E D C

V_LFOR-C

V_HPrimers

Oligo dT HO-TTTTTTTT(T)_n
 3' 5'

V_HBACK HO-----OH
 5' 3'

V_HFOR HO-----J_H for
 3' 5'

V_LPrimers

J_{kappa} for HO-----5'
 3'

V_LBACK HO-----OH
 5' 3'

V_LLinkers HO-----J_{kappa} for E D C
 3' 5'

V_LFOR-C HO-----C
 3' 5'

FIG. 7

Searching a recombinant antibody library

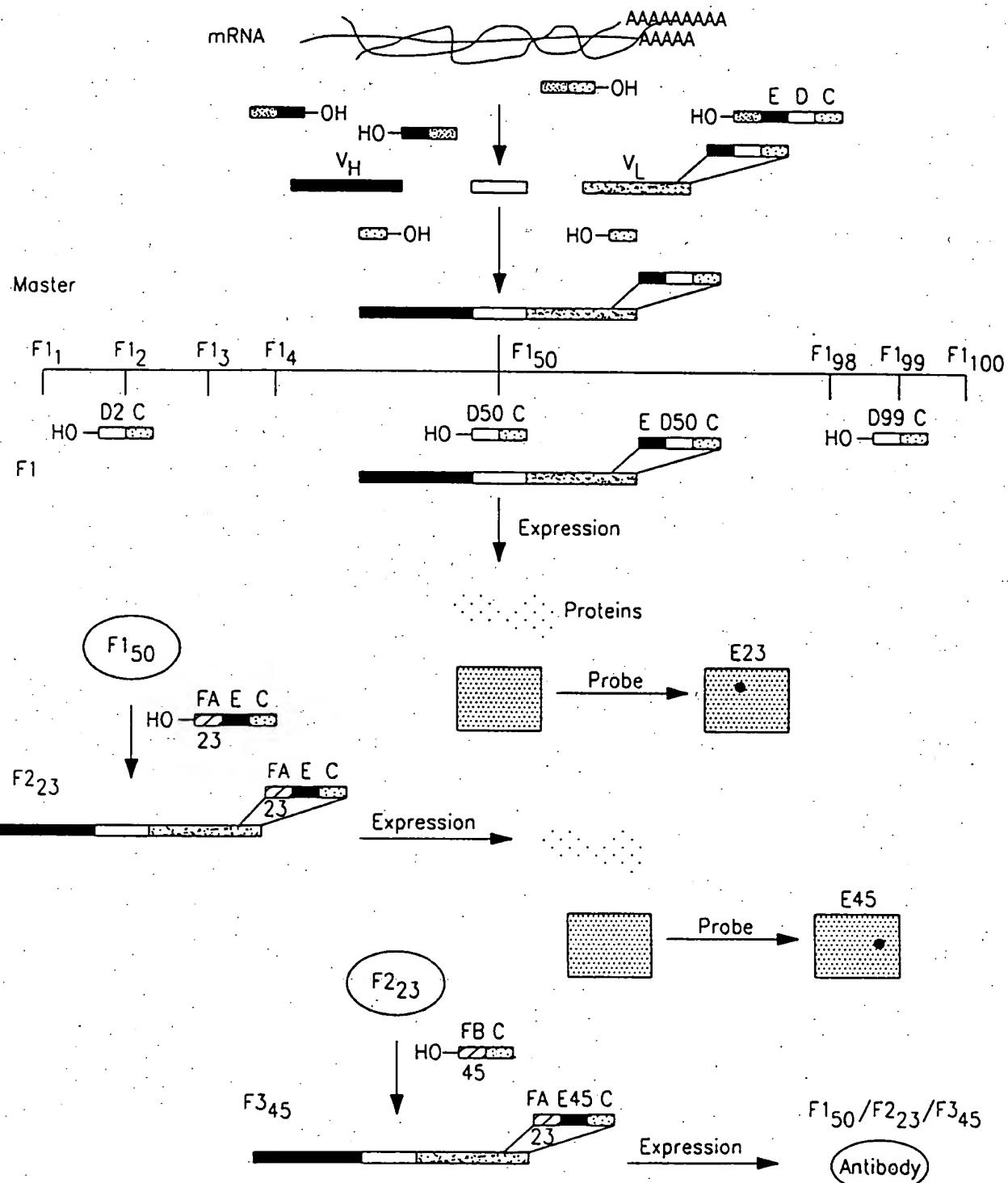


FIG. 8

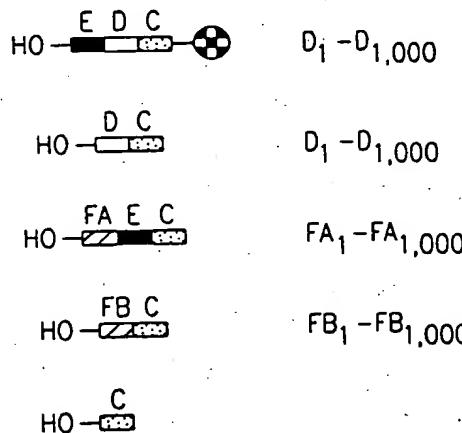
Title: **COLLECTIONS OF BINDING PROTEINS AND TAGS
AND USES THEREOF FOR NESTED SORTING AND
HIGH THROUGHPUT SCREENING.**

Applicant: Ault-Riche *et al.*
Serial No. 09/910,120 Filed: July 18, 2001
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Physical elements to include in the kits and combinations

• *Anti-tag Arrays™*

• Primer sets



• Readers

• Software

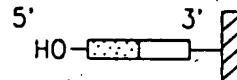
FIG. 9

Making the V_{LFOR} primers: Solid phase synthesis

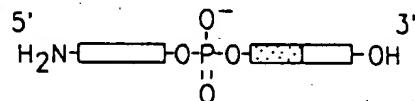


J_{kappa} for Epitope D Common

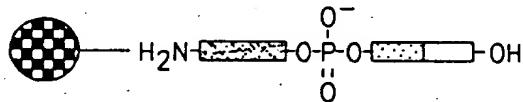
1. Synthesize oligo on solid support



2. Add aminolink prior to cleavage



3. Couple to tosyl activated magnetic beads



4. Extended by hybridizing with DNA probe and ligating

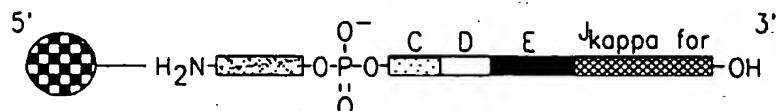
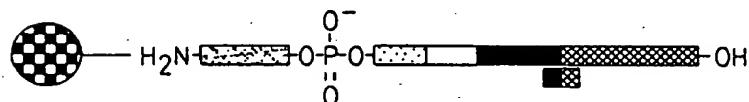
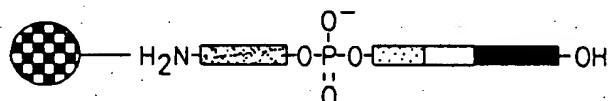
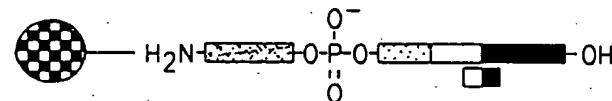
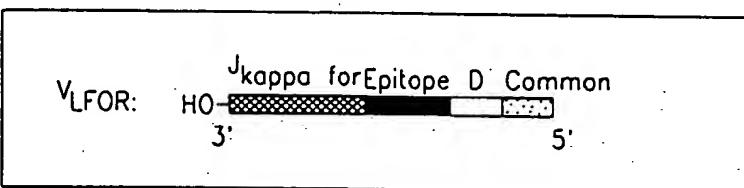


FIG. 10

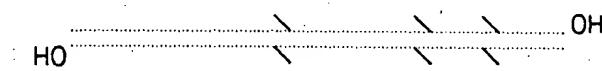
Making the V_{LEFOR} primers: Overlapping hybridization



J_{κ} for Epitope D Common

- Synthesize 4,028 different oligos:
(26 for J_{kappa} for : 2,000 for Epitope, 2,000 for D; 2 for Common

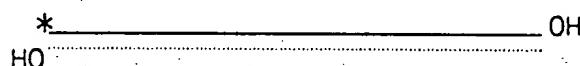
2. Assemble oligos for + and - strands of the different regions



3. Ligase the assembled oligos



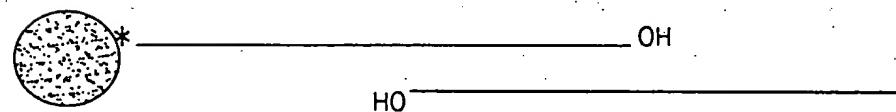
4. 1st strand synthesis with biotinylated primer



• 2nd strand synthesis with non-biotinylated primer.



6. Bind to avidin coated magnetic beads and then denature



7. Purify non-biotinylated ssDNA

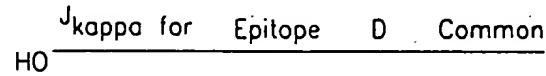


FIG. I

Building the collection of antibody/tag pairs: Hybridoma screening

Stable hybridoma cells

Isolated monoclonals
grown in 96-well plates
(quantify Abs in culture
supernatants by ELISA)

Purified antibodies
(purify with protein L plates;
quantify purified antibodies by ELISA)

Individual protein
preparations

Pooled protein
preparations

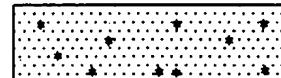
Array onto filter
(up to 10,000 per filter)



Bind purified antibodies to
magnetic beads coated with
anti-mouse Ig mAb (Dynal)

Pan a random disulfide-constrained
phage display library against the beads
(4 rounds with plate amplifications)

Bind enriched phage library to filter;
Stain with anti-phage mAb-HRP;
Image with CCD-based system



Cut out best spots
Recover and propagate phage
(or PCR amplify DNA)

Sequence DNA to
identify epitope tags

FIG. 12

Title: **COLLECTIONS OF BINDING PROTEINS AND TAGS
AND USES THEREOF FOR NESTED SORTING AND
HIGH THROUGHPUT SCREENING.**

Applicant: Ault-Riche *et al.*

Serial No. 09/910,120 Filed: July 18, 2001

Our Docket No.: 25885-1751

Table 3 Primers for PCR Amplification of Human Antibody Variable Regions (V genes)

1. V gene primary PCR

A. Human VH back primers (sense)

HuVH1aBACK	5'-CAG GTG CAG CTG GTG CAG TCT GG-3'
HuVH2aBACK	5'-CAG GTC AAC TTA AGG GAG TCT GG-3'
HuVH3aBACK	5'-GAG GTG CAG CTG GTG GAG TCT GG-3'
HuVH4aBACK	5'-CAG GTG CAG CTG CAG GAG TCG GG-3'
HuVH5aBACK	5'-GAG GTG CAG CTG TTG CAG TCT GC-3'
HuVH6aBACK	5'-CAG GTC CAG CTG CAG TCA GG-3'

B. Human JH forward primers (anti-sense)

HuJH1-2FOR	5'-TGA GGA GAC GGT GAC CAG GGT GCC-3'
HuJH3FOR	5'-TGA AGA GAC GGT GAC CAT TGT CCC-3'
HuJH4-5FOR	5'-TGA GGA GAC GGT GAC CAG GGT TCC-3'
HuJH6FOR	5'-TGA GGA GAC GGT GAC CGT GGT CCC-3'

C. Human V kappa back primers (sense)

HuV κ 1aBACK	5'-GAC ATC CAG ATG ACC CAG TCT CC-3'
HuV κ 2aBACK	5'-GAT GTT GTG ATG ACT CAG TCT CC-3'
HuV κ 3aBACK	5'-GAA ATT GTG TTG ACG CAG TCT CC-3'
HuV κ 4aBACK	5'-GAC ATC GTG ATG ACC CAG TCT CC-3'
HuV κ 5aBACK	5'-GAA ACG ACA CTC ACG CAG TCT CC-3'
HuV κ 6aBACK	5'-GAA ATT GTG CTG ACT CAG TCT CC-3'

C. Human V lambda back primers (sense)

HuV λ 1BACK	5'-CAG TCT GTG TTG ACG CAG CCG CC-3'
HuV λ 2BACK	5'-CAG TCT GCC CTG ACT CAG CCT GC-3'
HuV λ 3aBACK	5'-TCC TAT GTG CTG ACT CAG CCA CC-3'
HuV λ 3bBACK	5'-TCT TCT GAG CTG ACT CAG GAC CC-3'
HuV λ 4BACK	5'-CAC GTT ATA CTG ACT CAA CCG CC-3'
HuV λ 5BACK	5'-CAG GCT GTG CTC ACT CAG CCG TC-3'
HuV λ 6BACK	5'-AAT TTT ATG CTG ACT CAG CCC CA-3'

D. Human J kappa forward primers (anti-sense)

HuJ κ 1FOR	5'-ACG TTT GAT TTC CAC CTT GGT CCC-3'
HuJ κ 2FOR	5'-ACG TTT GAT CTC CAG CTT GGT CCC-3'
HuJ κ 3FOR	5'-ACG TTT GAT ATC CAC TTT GGT CCC-3'
HuJ κ 4FOR	5'-ACG TTT GAT CTC CAC CTT GGT CCC-3'
HuJ κ 5FOR	5'-ACG TTT AAT CTC CAG TCG TGT CCC-3'

D. Human J. lambda forward primers (anti-sense)

HuJ λ 1FOR	5'-ACC TAG GAC GGT GAC CTT GGT CCC-3'
HuJ λ 2-3FOR	5'-ACC TAG GAC GGT CAG CTT GGT CCC-3'
HuJ λ 4-5FOR	5'-ACC TAA AAC GGT GAG CTG GGT CCC-3'

FIG. 13A

Title: COLLECTIONS OF BINDING PROTEINS AND TAGS
AND USES THEREOF FOR NESTED SORTING AND
HIGH THROUGHPUT SCREENING.
Att: Ault-Riche *et al.*
Serial No. 09/910,120 Filed: July 18, 2001
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2. Linker fragment PCR

F. Reverse JH for scFv linker (sense)

—FR4 heavy —————— linker
 RHuJH1-2 5'-GC ACC CTG GTC ACC GTC TCC TCA GGT GG-3'
 RHuJH3 5'-GG ACA ATG GTC ACC GTC TCT TCA GGT GG-3'
 RHuJH4-5 5'-GA ACC CTG GTC ACC GTC TCC TCA GGT GG-3'
 RHuJH6 5'-GG ACC ACG GTC ACC GTC TCC TCA GGT GG-3'

F. Reverse Vk for scFv linker (anti-sense)

—FR1 light —————— linker
 RHuVkl1aBACKFv 5'-GG AGA CTG GGT CAT CTG GAT GTC CGA TCC GCC-3'
 RHuVk2aBACKFv 5'-GG AGA CTG AGT CAT CAC AAC ATC CGA TCC GCC-3'
 RHuVk3aBACKFv 5'-GG AGA CTG CGT CAA CAC AAT TTC CGA TCC GCC-3'
 RHuVk4aBACKFv 5'-GG AGA CTG GGT CAT CAC GAT GTC CGA TCC GCC-3'
 RHuVk5aBACKFv 5'-GG AGA CTG CGT GAG TGT CGT TTC CGA TCC GCC-3'
 RHuVk6aBACKFv 5'-GG AGA CTG AGT CAG CAC AAT TTC CGA TCC GCC-3'

F. Reverse Vλ for scFv linker (anti-sense)

—FR1 light —————— linker
 RHuVλ1BACK1Fv 5'-GG CGG CTG CGT CAA CAC AGA CTG CGA TCC GCC ACC GCC AGA G-3'
 RHuVλ2BACK2Fv 5'-GC AGG CTG AGT CAG AGC AGA CTG CGA TCC GCC ACC GCC AGA G-3'
 RHuVλ3BACK3aFv 5'-GG TGG CTG AGT CAG CAC ATA GGA CGA TCC GCC ACC GCC AGA G-3'
 RHuVλ4BACK3bFv 5'-GG GTC CTG AGT CAG CTC AGA AGA CGA TCC GCC ACC GCC AGA G-3'
 RHuVλ5BACK4Fv 5'-GG CGG TTG AGT CAG TAT AAC GTG CGA TCC GCC ACC GCC AGA G-3'
 RHuVλ6BACK5Fv 5'-GA CGG CTG AGT CAG CAC AGA CTG CGA TCC GCC ACC GCC AGA G-3'
 RHuVλ7BACK6Fv 5'-TG GGG CTG AGT CAG CAT AAA ATT CGA TCC GCC ACC GCC AGA G-3'

3. Pull-through primers for introduction of restriction sites*

G. Human VH back (Sfi) primers (sense)

HuVH1aBACKSfi —————— FR1 heavy
 5'-GTC CTC GCA ACT GCG GCC CAG CGG GCC ATG GCC CAG GTG CAG CTG GTG CAG TCT GG-3'
 HuVH2aBACKSfi
 5'-GTC CTC GCA ACT GCG GCC CAG CGG GCC ATG GCC CAG GTC AAC TTA AGG GAG TCT GG-3'
 HuVH3aBACKSfi
 5'-GTC CTC GCA ACT GCG GCC CAG CGG GCC ATG GCC GAG GTG CAG CTG GTG GAG TCT GG-3'
 HuVH4aBACKSfi
 5'-GTC CTC GCA ACT GCG GCC CAG CGG GCC ATG GCC CAG GTG CAG CTG CAG GAG TCG GG-3'
 HuVH5aBACKSfi
 5'-GTC CTC GCA ACT GCG GCC CAG CGG GCC ATG GCC CAG GTG CAG CTG TTG CAG TCT GC-3'
 HuVH6aBACKSfi
 5'-GTC CTC GCA ACT GCG GCC CAG CGG GCC ATG GCC CAG GTA CAG CTG CAG CAG TCA GG-3'

H. Human J kappa forward (Not) primers (anti-sense)

HuJk1FORNot —————— FR4 light
 5'-GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT GAT TTC CAC CTT GGT CCC-3'
 HuJk2FORNot
 5'-GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT GAT CTC CAC CTT GGT CCC-3'

H. Human J kappa forward (Not) primers (anti-sense)(continued)

HuJk3FORNot —————— FR4 light
 5'-GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT GAT ATC CAC TTT GGT CCC-3'
 HuJk4FORNot
 5'-GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT GAT CTC CAC CTT GGT CCC-3'
 HuJk5FORNot
 5'-GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT AAT CTC CAG TCG TGT CCC-3'

H. Human J lambda forward (Not) primers (anti-sense)

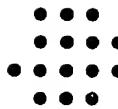
HuJ11FORNOT —————— FR4 light
 5'-GAG TCA TTC TCG ACT TGC GGC CGC ACC TAG GAC GGT GAC CTT GGT CCC-3'
 HuJ12-3FORNOT
 5'-GAG TCA TTC TCG ACT TGC GGC CGC ACC TAG GAC GGT CAG CTT GGT CCC-3'
 HuJ14-5FORNOT
 5'-GAG TCA TTC TCG ACT TGC GGC CGC ACC TAA AAC GGT GAC CTT GGT CCC-3'

*Recognition site for restriction enzyme is underlined.

FIG. 13B

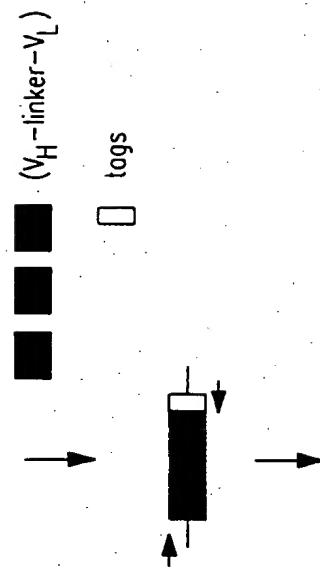
Title: **COLLECTIONS OF BINDING PROTEINS AND TAGS
AND USES THEREOF FOR NESTED SORTING AND
HIGH THROUGHPUT SCREENING.**

Applicant: Ault-Riche *et al.*
Serial No. 09/910,120 Filed: July 18, 2001
Our Docket No.: 25885-1751



Step 1

Tag and assemble immunoglobulin genes



Create 1,000 sub-libraries by separate PCR amplification
reactions using tag-specific PCR primers

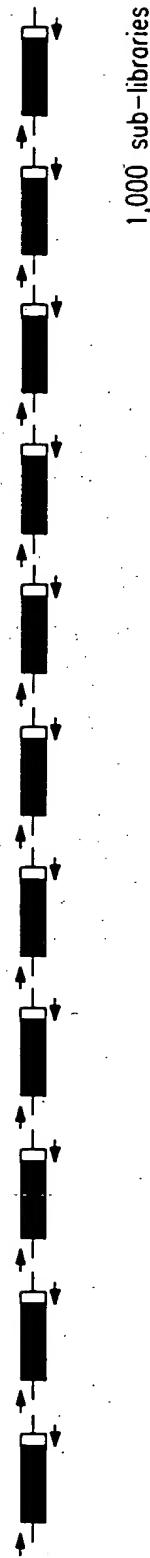
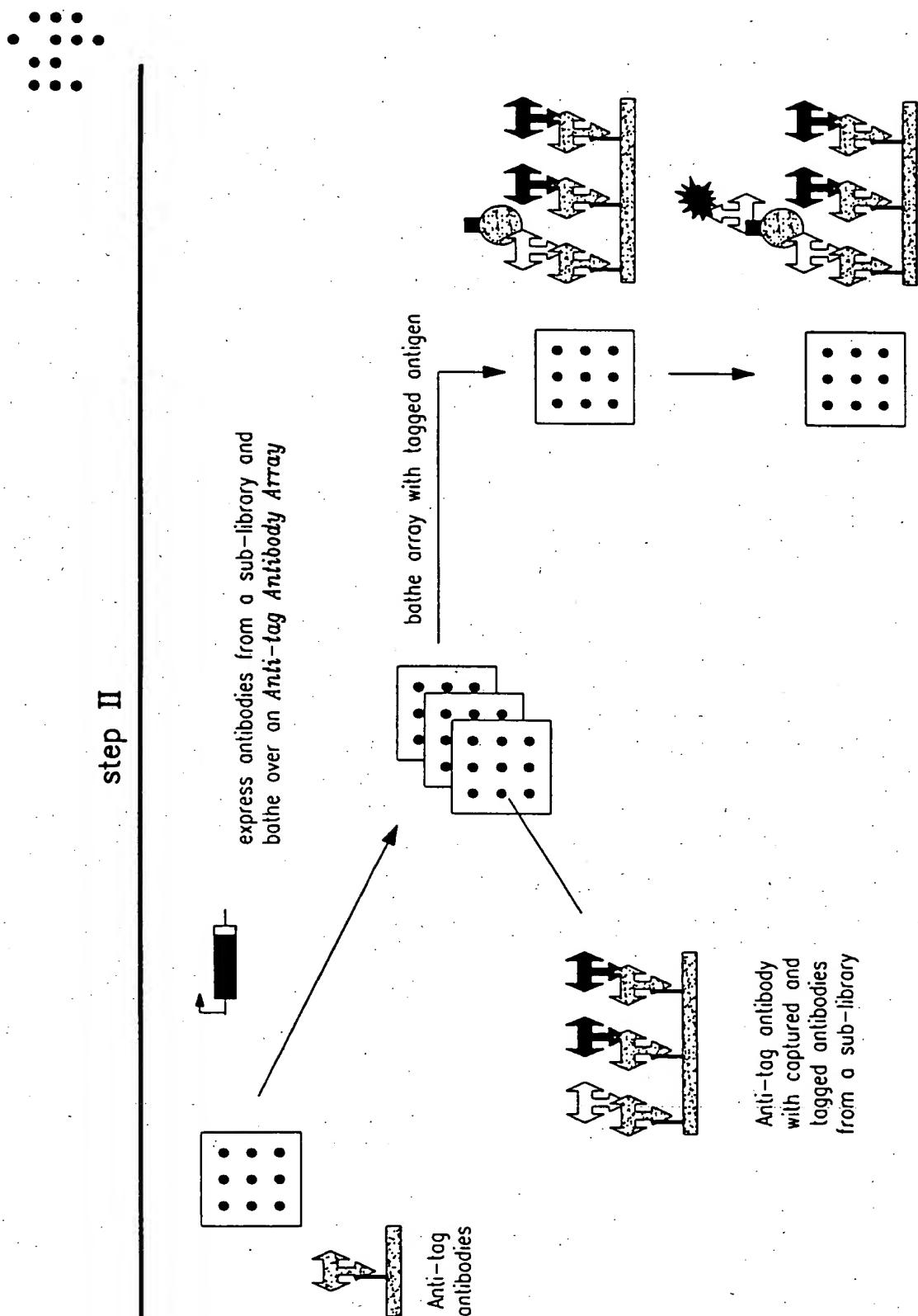


FIG. 14A

Title: **COLLECTIONS OF BINDING PROTEINS AND TAGS
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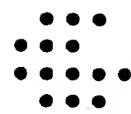


ID spot containing the antigen with a labeled developing Ab

FIG. 14B

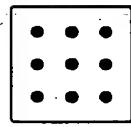
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Step III

Amplify the antibody genes from the identified sub-library using tag-specific PCR primers

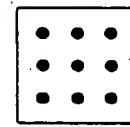


If the starting diversity of the master library was 1,000,000,000 then each spot in this array will have 1,000 different types of rAbs

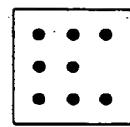
Express and purify the antibodies



Re-distribute over an Anti-tag Antibody Array



If the starting diversity of the master library was 1,000,000,000 then each spot in this array will have a single type of rAb

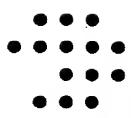


Re-survey to ID the antibody of interest

FIG. 14C

Title: **COLLECTIONS OF BINDING PROTEINS AND TAGS
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summary

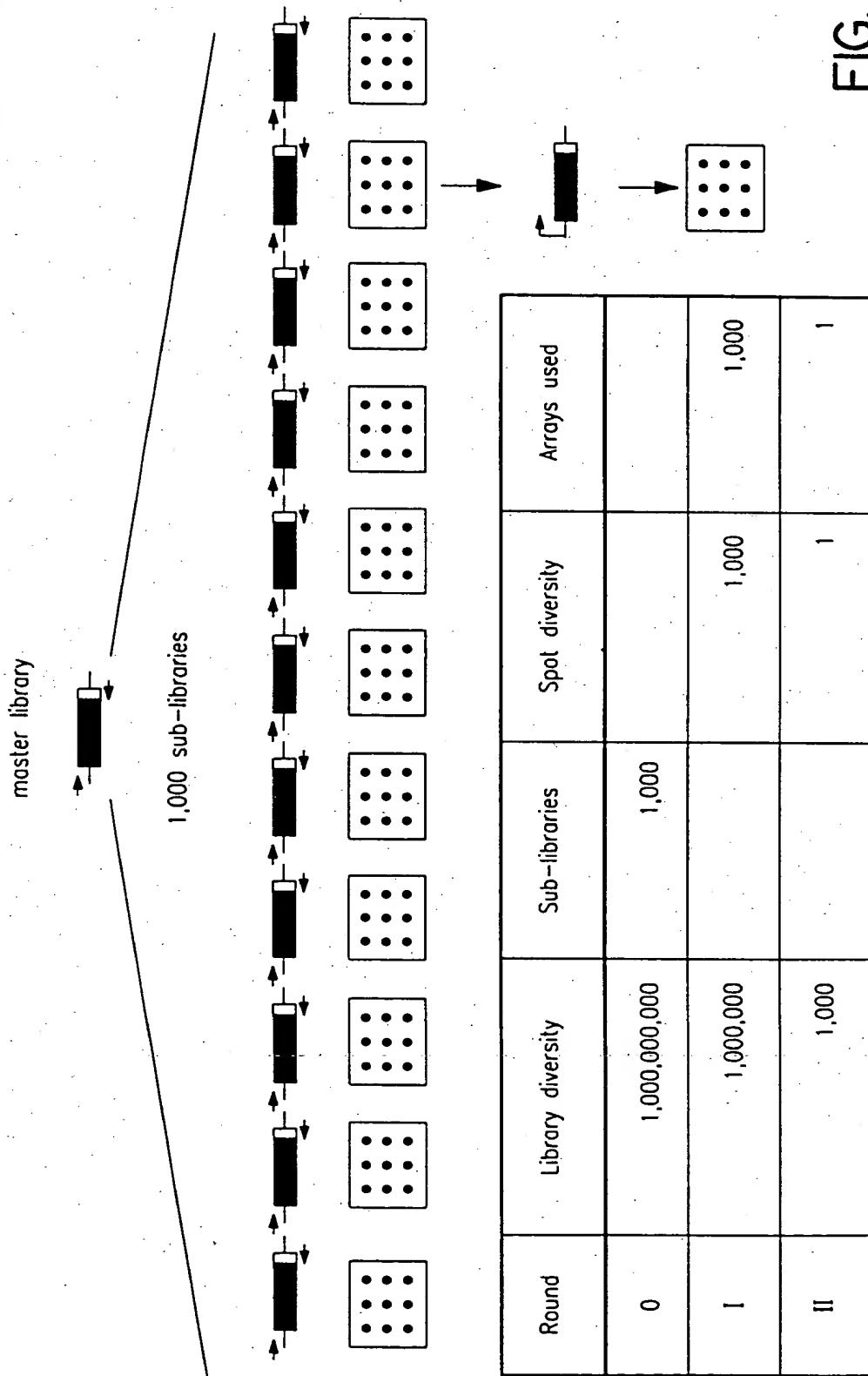
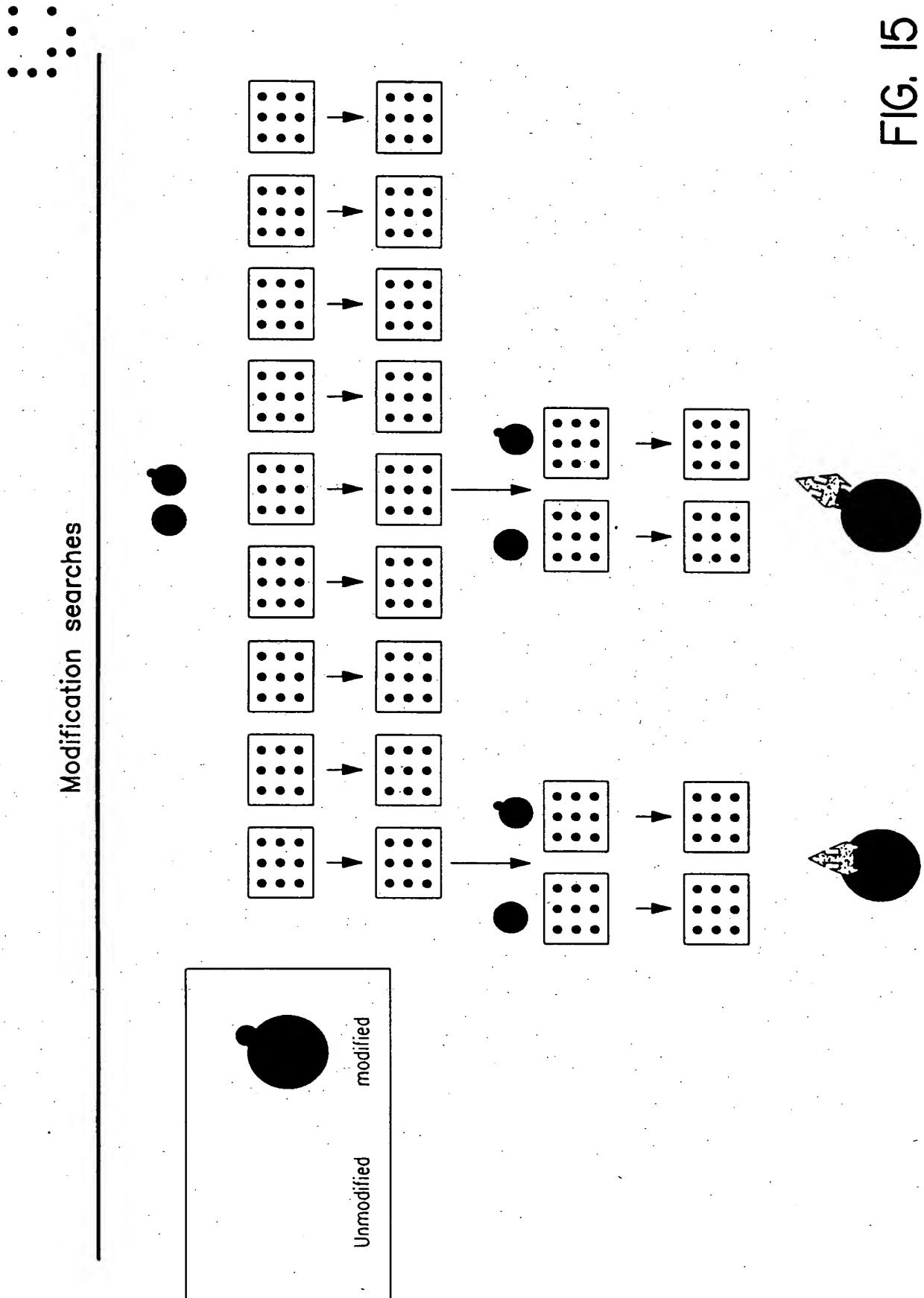


FIG. 14D

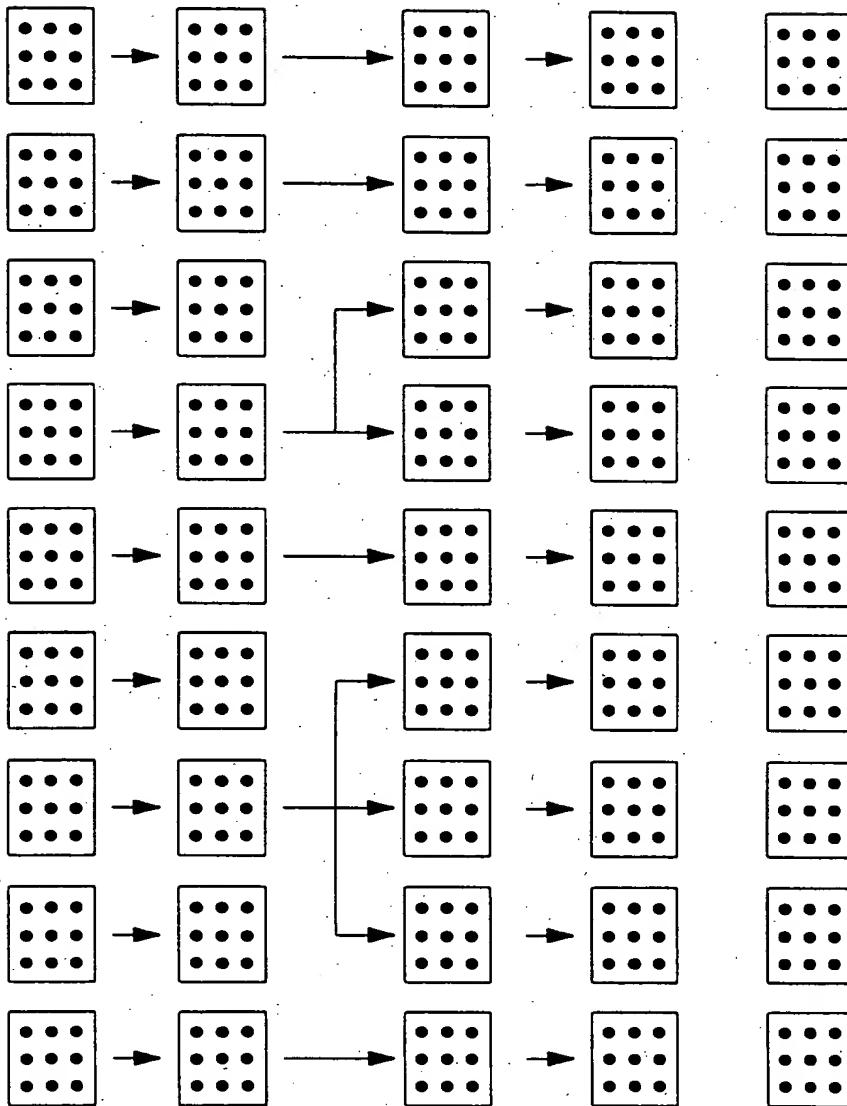
Modification searches



Title: **COLLECTIONS OF BINDING PROTEINS AND TAGS
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Simultaneous searches**Round Arrays Bait Probe****I 1,000 Abs Abs****II 1,000 Abs Abs****III $\frac{1,000 \text{ Abs Abs}}{3,000}$** **3 Arrays per Ag****FIG. 16**

Title: **COLLECTIONS OF BINDING PROTEINS AND TAGS
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Protein interaction mapping

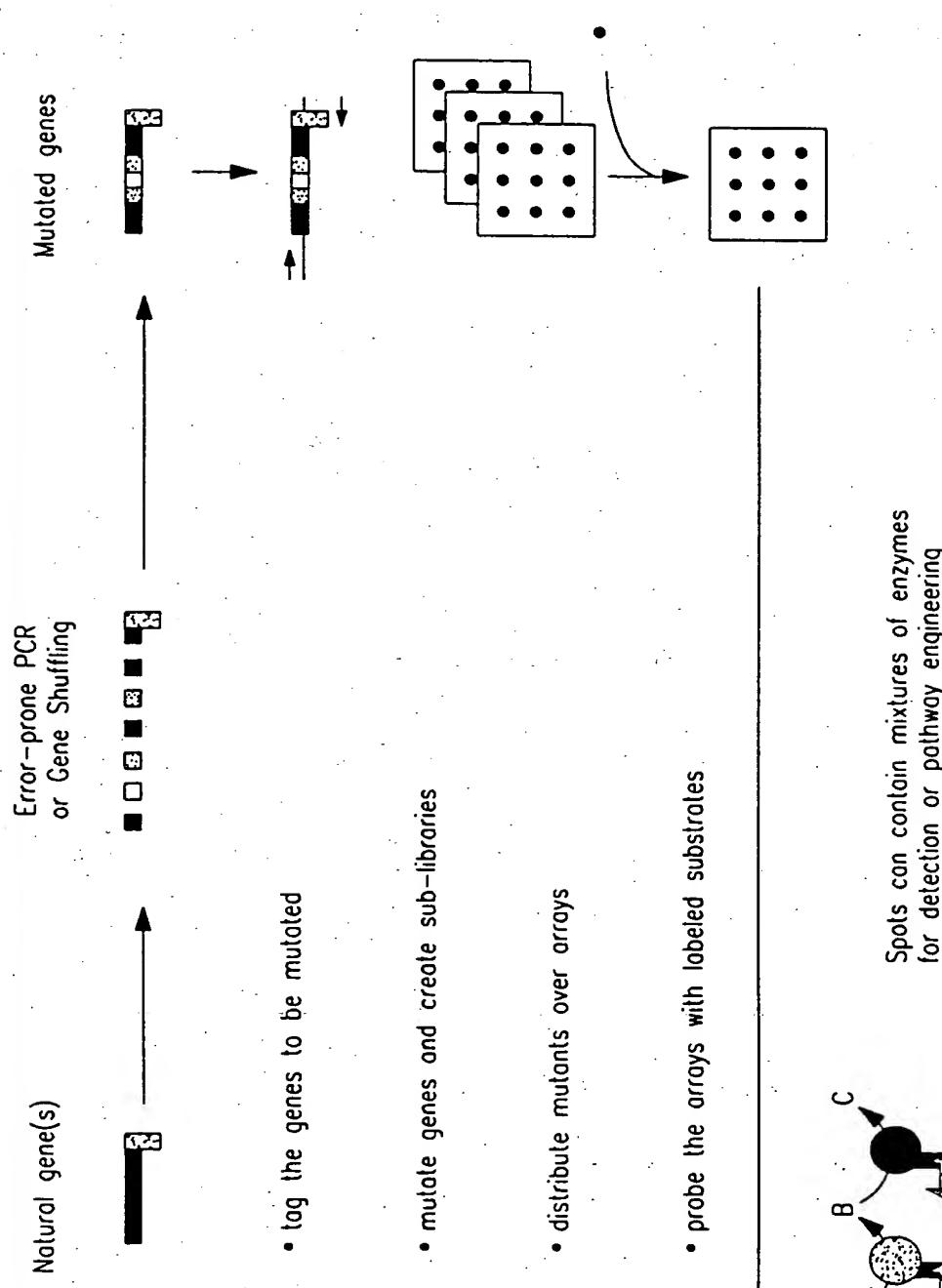
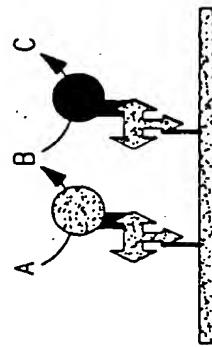


FIG. 17



Protein interaction mapping

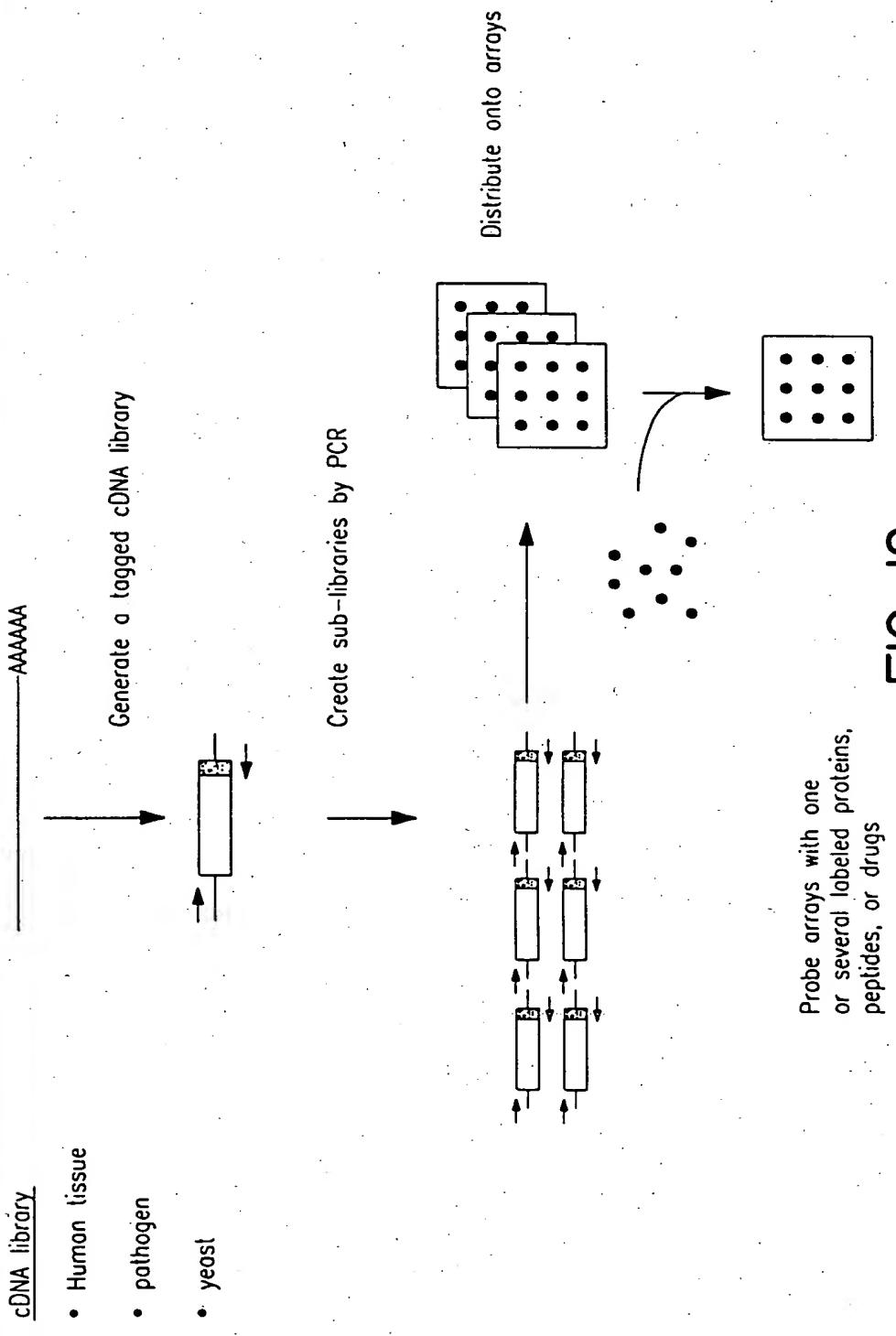


FIG. 18

Title: **COLLECTIONS OF BINDING PROTEINS AND TAGS
AND USES THEREOF FOR NESTED SORTING AND
HIGH THROUGHPUT SCREENING.**
Inventor: Ault-Riche *et al.*
Serial No. 09/910,120 Filed: July 18, 2001
Our Docket No.: 25885-1751

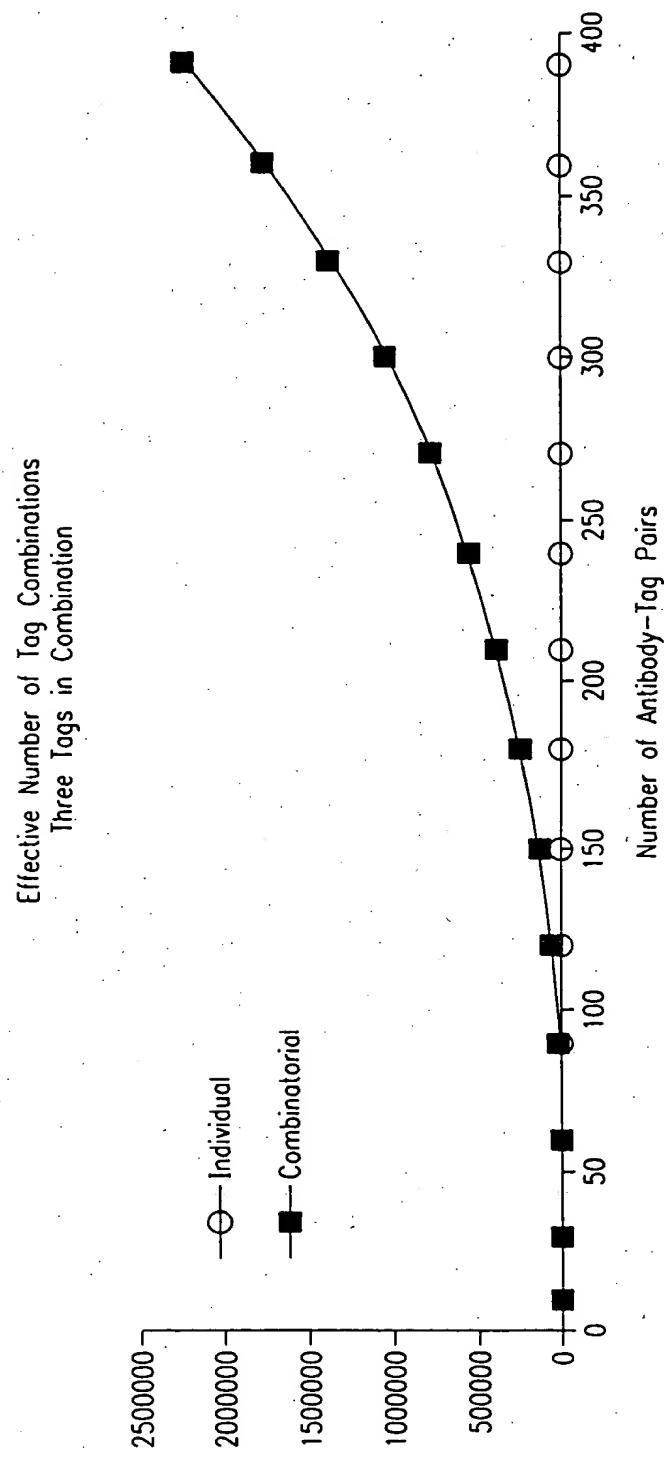


FIG. 19